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HEPATOPROTECTIVE AND ANTIOXIDANT EFFECTS OF METHANOL EXTRACT OF SOURSOP (*Annona muricata*) SEEDS ON ALLOXAN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

This study aimed at investigating hypoglycemic, hepatoprotective and antioxidant effects of *Annona muricata* seeds methanol extract (AMSME). Hepatoprotective and antioxidant effects were examined using alloxan induced diabetic rats. Twenty-four (24) Wistar rats were grouped into five: normal control group, positive control group were induced with alloxan 125 mg/kg body weight and treated with Glibenclamide, negative control group that were induced but never treated and two treatment groups that were induced and treated with *Annona muricata* extract at a dose of 50 and 100 mg/kg body weight. The blood glucose levels were determined before and after alloxan induction during the period of 28 days treatment using glucometer. At the end of the study, the animals were sacrificed through ocular puncture for liver function tests examination. Blood glucose and liver function parameters in all treated groups were significantly decreased ($p<0.05$) when compared with the control. From the result, blood glucose level in all the groups that received *Annona muricata* extracts of 100 mg/kg body weight (99.7±0.03 mg/dl) significantly decreased ($p<0.05$) when compared to the positive control (160.2±0.22 mg/dl). Activity of all the marker enzymes (AST, ALT, ALP, TP, Albumin, Globulin) showed significant decreases in all the groups treated with *Annona muricata* extract as compared with the untreated negative control showing no sign of liver damage, signifying the hepatoprotective effect of *Annona muricata* extract at all doses. The results also show reduced MDA levels while

restoring SOD, and CAT activities content. The results showed that the antidiabetic property of *Annona muricata* methanol extract could be expound by the antioxidant and protective action on the pancreatic β-cells that in turn enhance glucose reactions that occur in the body.

Keywords: Alloxan, *Annona muricata*, Blood glucose level, Hepatoprotective, Liver function test

Abbreviations: SARS, severe acute respiratory syndrome; AIDS, acquired immunodeficiency syndrome; ANS, autonomic nervous system; AMSME, Annona muricata seed methanol extract; mg, milligram; kg, kilogram; ml, milliliter; AMSE, Annona muricata seed extract; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; TP, total proteins; dl, deciliter; WHO, world health organization; IDF, international diabetes federation; mmol/L, millimoles per liter; SPSS, statistical package for the social sciences; ANOVA, analysis of variance; SEM, standard error mean, H_2O, water, CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde; β-cells, Beta cells.

INTRODUCTION

Diabetes mellitus is a metabolic condition which is characterized by disturbance in the breaking down of carbohydrates, lipids and proteins as a result of moderate or average insulin deficiency [1]. Diabetes is not a single disorder; it is symptom disease that has a peculiar feature of high blood glucose level due to imbalanced insulin production [1].

Recently synthetic antidiabetic agents are linked with disadvantages such as resistance and serious side effects: heart disease, liver toxicity, kidney damage, stroke, abdominal discomfort, flatulence and nerve damage [2]. This has led to many people using medicine plants to treat diabetes disorder, though most of the medicinal plants do not have scientific assertion to prove their usage and efficacious actions [3]. Some of the plants that are used include the following: *Ageratum conyzoides*, *Blighia sapida*, *Calotropis procera*, *Ficus exasperate*, *Carica papaya and Annona muricata* [4].

Sour-sop seeds are very nutrient-densed and provide a good amount of acetogenins containing compounds namely bulatacin, asimisin and squamosin [5]. Phytochemical analysis of *Annona muricata* (Soursop) *leaf* extract revealed the presence of some secondary metabolites like tannins, steroids, and cardiac glycosides. Soursop leaf also contains several plant compounds

that act as antioxidants, including luteolin, quercetin and tangeretin. The nutrients in sour-sop leaves are believed to reduce blood sugar level in the normal range that is very useful for diabetics' management and treatment [5]. Studies have shown that *Annona muricata* leaves had antihyperglycemic activity and showed regeneration of pancreatic islet [6].

Plate 1: Sour-sop fruit and seed [7]

Plants have always found a place in the existence of man as they play a major significant role either as food, medicine or by products for human utilization [8]. Man's life had depended on plants not just for food but as key or starting organisms in the food chain of living things. These and many more have made it a fixed characteristic of man to unravel which plants are nutritious, medicinal and perhaps for the production of goods and services [8]. Additionally, plants are playing unrestrained roles in the maintenance of human health because of their medicinal values. The decoction of various plants part like the stem, seed, roots and leaves have been employed in the treatment of ringworm, itch, eczema, helminthiasis, cut wound, boils, foul ulcer, diabetes and various intestinal troubles in rural areas [8].

The exploration of a single bioactive compound of a medicinal plant has given rise to source of new drugs. Some of these drugs have chemotherapeutic effects and are used against most life threatening diseases like cancer, AIDS, SARS, diabetes etc. the wide range of biological activities of medicinal plants are studied worldwide [9].

Prolong implication of diabetes could lead to progressive diseases of renal dysfunction and autonomic nervous system (ANS) failure [10].

Due to the numerous medical and pharmacological properties of *Annona muricata for* the management of ailments, this study was aimed at evaluating the possible hypoglycemic and antioxidant effects of methanol extract of *Annona muricata* seed on alloxan-induced wistar rats.

MATERIALS AND METHODOLOGY

Plant Materials/Extraction

Annona muricata fruits were bought from Orie-Ugba market in Umuahia North Local Government Area of Abia State, Nigeria. The seeds were collected, washed and oven dried. It was weighed and milled into powdered form 250g. The powdered seeds were soaked in methanol and distilled water in the ratio of 80:20 respectively and left to stand for 3 days with occasional shaking. This was filtered using Whatman No.1 filter paper. The filtrate was subsequently evaporated to obtain the dry matter by using a rotary evaporator under reduced pressure at 40^{o}C.

Experimental animals

Twenty (24) healthy male wistar rats weighing between 100-120g, obtained from Ogive Integrated farm house, Aba, Abia State, were for the study. The animals on arrival were weighed to obtain initial weight and were acclimatized for 14 days in the animal house of Biochemistry Department, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, The animals were brought to daylight for 12 hours under normal tropical weather conditions with access to standard food and water till the end of the research which lasted for 28 days.

Ethical consideration

Throughout the experiment, all the rats were housed at 25^{o}C in clean cages under normal daylight/day humid conditions. The rats freely feed (vital feed pellets) and were given running (tap) water and were made available throughout the process according to the guidelines approved by the Departmental committee on animal use, Michael Okpara University of Agriculture, Umudike on handling of experimental rats.

Induction of Diabetes

At the end of acclimatization, the animals were allowed to fast and then diabetes was induced by intra-peritoneal (IP) injection of 120mg/kg body weight of alloxan monohydrate solution. Animals with blood glucose level higher than 150mg/dl were considered diabetic after 3 days of induction using fasting blood sugar method and were selected for the study.

Determination of Blood Glucose

The level of blood glucose was determined using glucose oxidase methods, by collecting 0.5ml of blood from the tail after a mild anesthesia using ether. Fasting blood sugar level was calculated and evaluated in mmol/L using a digital glucometer (Accu-check® Advantage, Roche Diagnostic, Germany). Animals were fasted for 16 hours prior to blood collection.

Experimental Design

After acclimatization, 20 male animals were chosen for further experimentation. These were divided into 5 groups with 4 animals each. The groups are thus:

Groups		Treatment
Group 1	Normal control	Feed + H_2O ad libitum
Group 2	Negative control	Alloxan + Feed + H_2O ad libitum
Group 3	Positive control	Alloxan+ Standard drug (Glibenclamide) + Feed + H_2O
Group 4	Annona muricata	Alloxan + 50mg/kg extract + Feed + H_2O ad libitum
Group 5	Annona muricata	Alloxan + 100mg/kg extract + Feed + H_2O ad libitum

Preparation of drugs

Glibenclamide (glyburide) 500mg solution was prepared by crushing the tablet in a glass mortal and dissolved in 1ml of distilled water to give 500mg/ml in stock solution. Glibenclamide (glyburide) was orally administered to the animals at a dose of 500mg/kg body weight.

Determination of blood glucose concentration (mg/dl)

The glucose concentration was measured using glucometer

Collection of blood sample and organs

The animals were fasted overnight and blood was collected through ocular puncture. Samples of blood were collected into sample bottles (plasma for hematological analysis).

Hepatic Indices

Serum AST and ALP activities were estimated by the method of Reitman and Frankel [12], using the Randox test kit. The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss [1]. The concentration of bilirubin was determined using the method of Jendrassik and Grof [1] as outlined in Randox test kit. Total protein estimation was determined by the method of Tietz [1] as stated in Randox test kit.

Determination of antioxidant

Determination of superoxide dismutase (SOD)

The Sun and Sigma method as described by Ian and Soon [11] was adopted.

Determination of catalase activity

Determination of catalase activity was according to the method of Sinha [12].

Malondialdehyde (MDA) level Determination

Lipid peroxidation was determined using formation of Malondialdehyde (MDA) and measured by thiobarbituric reactive (TBARS) method as described by Onkawa et al. [13].

Statistical Analysis

Statistical analysis of data was carried out using one way analysis of variance (ANOVA) Statistical package for social sciences (SPSS) version 20.0. The analysis data was reported as mean ± standard error of mean (SEM). Significant difference using Tukey's Post Hoc test was accepted at 95% confidence level of probability i.e. at $p< 0.05$.

RESULTS AND DISCUSSION

Effects of Glucose levels

Table 1 shows the treatment of *Annona muricata* (seed) extract at the doses of 50mg/kg and 100mg/kg respectively significantly (P>0.05) decreased the level of blood glucose as compared to control. However, in comparison with positive control, glibenclamide, there was a significant (P>0.05) decrease in the blood glucose levels. However, the dose 100mg/kg had the maximum glucose reducing effect as compared with the control.

Effects of Serum Enzymes Concentration

Table 2 showed that the mean serum enzymes (AST, ALT, ALP, TP, Albumin, Globulin) concentration of *Annona muricata* (seed) extract at the doses of 50 mg/kg and 100 mg/kg respectively, significantly (P>0.05) decreased the serum enzyme concentration when compared to the control. The result showed that the extract has hepatoprotective effect on the treated rats.

Table 3 showed that the activity of all the marker enzymes (TP, Albumin, Globulin) concentration of *Annona muricata* (seed) extract at the doses of 50 mg/kg and 100 mg/kg respectively significantly (P>0.05) decreased the biomarker enzymes concentration when

compared to the control. The result showed that the extract has hepatoprotective effect on the treated rats.

Effects of Malondialdehyde level

Table 4 shows the comparison of the positive control group and the negative control group of non-significant increase of MAD by 35.32 ± 5.98. Treatment with the extract shows a dose-dependent decrease in MDA levels and is significantly lower than the negative control group. Also, the extract group of 50mg/kg showed the least level of MDA concentration. However, the normal control group (27.33 ± 2.82) shows a relative decrease in MDA level when compared to the negative control group (35.32 ± 5.98).

Effects of Superoxide Dismutase level

Table 4 shows the normal control group shows high SOD (2.13 ± 0.25*) level when compared to the negative control group (0.19 ± 0.01) is significant. Also for the positive control (1.90 ± 0.13), the levels of SOD are relatively higher when in comparison with the negative control group (0.19 ± 0.01). The treatment group 100mg/kg shows elevated amount of SOD when compared to the negative control (0.19 ± 0.01) and extract group of 50mg/kg.

Effects of CATALASE

Table 4 shows the catalase level in normal control group (68.87 ± 4.58*) is significantly higher when compared to the negative control group (54.82 ± 0.97). For the treatment groups, there is a dose-dependent increased significantly in catalase level as compared with the negative control group (54.82 ± 0.97). The positive control group (64.52 ± 4.31) also showed relative higher catalase amounts than the negative control group (54.82 ± 0.97).

Table 1: Comparison of mean glucose level in the *Annona muricata seed* extract in normal control, positive and negative control for 28 days

Group/Treatment	WEEK 0	DAY 7	DAY 14	DAY 21	DAY 28
Group I (Control)	69.0±0.03	68.3±0.13	65.0 ± 0.10	64.0±1.11	62.1±0.01
Group II (Diabetic untreated)	364.1±0.05	336.2±2.11	371.3±1.12	392.0±0.21	398.0±0.07
Group III (2 mg/kg Glibenclamide)	306.1±0.12	280.1±1.11*	203.7±0.21*	179.3±0.07*	160.2±0.22*

Group IV (Sour-sop 50 mg/kg)	316.5±0.09	253.0±0.10*	200.0±1.02*	171.0±0.13*	151.6±0.11*
Group V (Sour-sop 100 mg/kg)	313.5±0.17	215.1±0.01*	195.0±1.04*	140.5±0.60*	99.7±0.03*

(Values are mean ± SD; n=5. Values are statistically significant *(P<0.05). Group 1, Normal control= feed and water only, group 2, Negative control = induced with Alloxan but were not treated, group 3, Positive control= received oral treatment with Glibenclamide, Group IV= received oral treatment with *Annona muricata* seed extract (50mg/kg), Group V=received oral treatment with *Annona muricata* seed extract (100mg/kg). The highest activity resides at 28 days of administration when compared to control).

Table 2: Comparison of mean AST, ALT and ALP levels in the *Annona muricata seed* extract treated groups with the negative control

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group I:Normal Control (feed + water)	46.07 ± 6.83*	12.27 ± 0.27*	17.91 ± 0.37*
Group II: Negative Control (Alloxan but not treated)	81.27 ± 2.78	56.60 ± 5.39	99.82 ± 2.27
Group III: Positive Control (Alloxan + Glibenclamide)	47.40 ± 2.86*	13.40 ± 1.40*	84.85 ± 7.39*
Group IV: Diabetic rats treated with extract of 50mg/kg	54.47 ± 2.14*	17.50 ± 0.50*	50.55 ± 1.42*
Group V: Diabetic rats treated with extract of 100mg/kg	47.60 ± 2.44*	19.80 ± 1.53*	63.86 ± 0.41*

(Mean ± SEM; *P<0.05= Values are significantly statistical compared to negative control, ns=not significant).

Table 3: Comparison of mean TP, Albumin and Globulin levels in the groups treated with extract for the Normal group, the positive and the negative control

Groups	TP (g/dl)	ALB (g/dl)	GLB (g/dl)
Group I:Normal Control (feed + water)	6.64 ± 0.42*	3.41 ± 0.02*	2.22 ± 0.40*
Group II: Negative Control (Alloxan but not treated)	5.53 ± 0.42	4.63 ± 0.15	3.75 ± 0.07

Group III: Positive Control (Alloxan + Glibenclamide)	6.15 ± 0.04*	3.00 ± 0.04*	2.902 ± 0.39*
Group IV: Diabetic rats treated with extract of 50mg/kg	5.72 ± 0.18	3.15 ± 0.21*	2.57 ± 0.39*
Group V: Diabetic rats treated with extract of 100mg/kg	6.13 ± 0.09*	3.33 ± 0.07*	2.81 ± 0.11*

(Mean ± SEM; *P<0.05= Values are significantly statistical compared to negative control, ns=not significant).

Table 4: Effects of methanol extract of *Annona muricata* seed on the organosomatic indices of alloxan-induced hyperglycemic rats

Group	MDA (nanomole/g protein)	SOD (U/g protein)	CAT (kU/L/g Protein)
GP 1 normal control	27.33 ± 2.82	2.13 ± 0.25*	68.87 ± 4.58*
GP 2 Negative control	35.32 ± 5.98	0.19 ± 0.01	54.82 ± 0.97
GP 3 Positive control	20.87 ± 0.72*	1.90 ± 0.13*	64.52 ± 4.31*
GP 4 SS 50 mg/kg	22.08 ± 1.16*	0.57 ± 0.03	62.34 ± 1.55*
GP 5 SS 100 mg/kg	28.98 ± 2.27	1.46 ± 0.03*	63.26 ± 0.75*

(Mean ± SEM; *p<0.05 when compared to the negative control group)

Medicinal plants are generally known and popular for a number of health benefits such as cardiovascular diseases or reducing the risk of cancer also due to their antioxidant activity. Medicinal plants have been the basis of treatment of various diseases in Africa traditional medicines as well as some other forms of treatment from diverse culture of the world.

Diabetes mellitus is a metabolic disorder in which there is an inability to complete dispose of glucose due to disturbances in insulin function resulting in glucosuria and hyperglycemia [14]. Diabetes mellitus is characterized by chronic hypercholesterolemia, hyperlipidemia and hepatic steatosis resulting from defects in insulin secretion, insulin action or both. The pharmacological treatment of disease started ages ago with the use of herbs [15].

Annona muricata seeds extract (AMSE) findings showed that AMSE extract is a generic medicine to balance glucose levels [1]. Also the findings showed some potentials for controlling diabetes through significant antihyperglycemic activity at a concentration of 100mg/kg when compared to the Glibenclamide (standard drug). The constituent of flavonoids in the AMSE reveals its different pharmacological and biological activities [16].

Flavonoids play a variety of biological activities in plants, flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, function as a signal molecules,

allelopathic compounds, phytoalexins, detoxifying agents, antimicrobial defensive compounds [16]. Considering these several studies, it can be concluded that the antidiabetic and antihyperglycemic activities of the methanolic seeds extract of *A.muricata* is mostly associated with contents in flavonoids.

Hyperglycaemia in diabetic disorder can reduce the levels of antioxidants and elevated free radical products [17]. These results showed that *Annona muricata* contain antioxidant properties. The present findings are in agreement with those of Adewole and Caxton- Martins [18], who reported the activities of antioxidant of *Annona muricata* aqueous extract in short-term treatment.

The Sour-sop extract induced a significant reduction in alloxan-induced diabetic wistar rats. It reaches the normal value at the dose of 100 mg/kg. It is a known fact that alloxan acts by destructing β-cells [19]. Thus the medicinal plant extract could act in this condition by improving peripheral glucose uptake.

The results above have highlighted the anti-diabetic, anti-hyperglycemic, hepatoprotective and anti-oxidant activity of the methanol extract of *A. muricata* seed in vivo.

CONCLUSION

Induction of oxidative stress is a major key process in the onset of diabetic complications. This study has shown that oral administration of the methanolic extract of *Annona muricata* seed significantly reduced blood glucose levels and also showed hepatoprotective and antioxidant effects on the diabetic wistar rat. This shows that the various phytochemicals present in the extract were to an extent effective in the treatment and management of diabetes and oxidative stress. Also, the actions of the extracts were dose-dependent which shows that the right amount and quantity should be considered before administration and use.

Conflict of interest

There was no conflict of interest declared by the authors

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